Supplemental information

Supplemental Figures

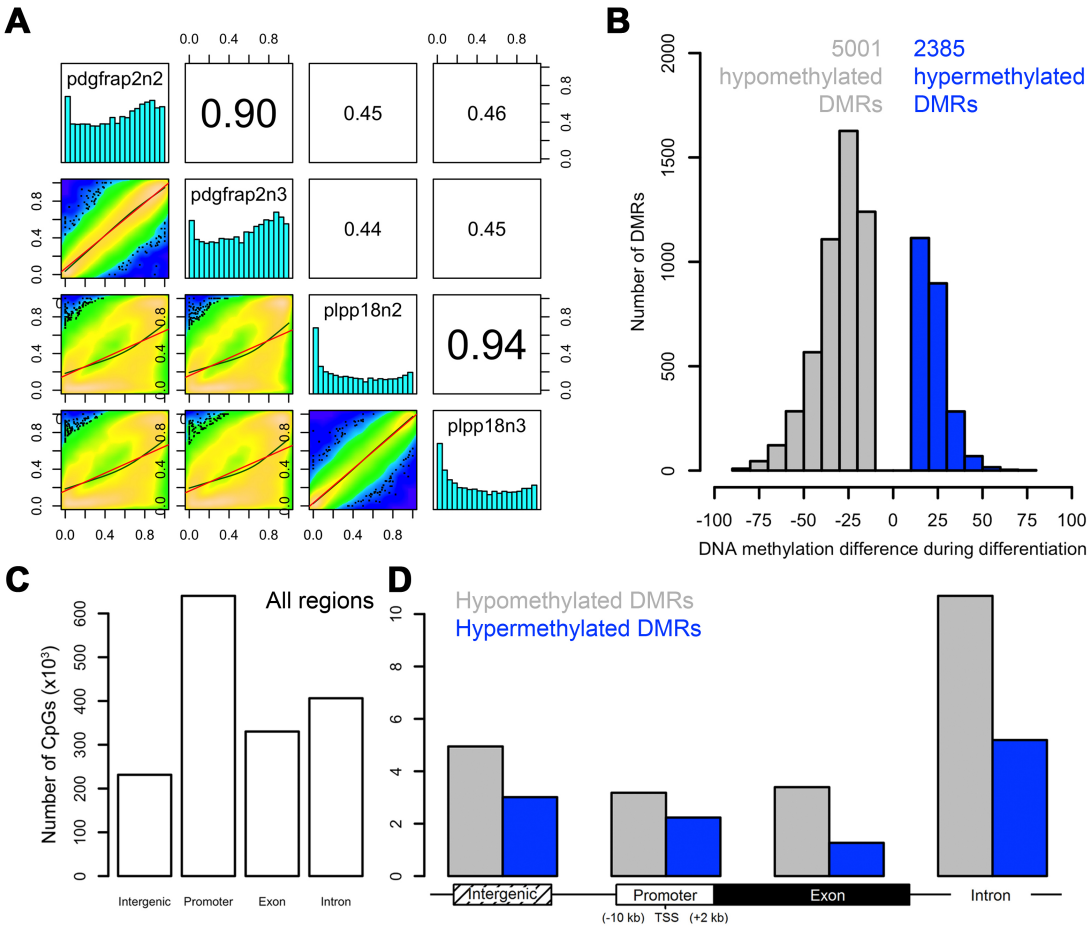
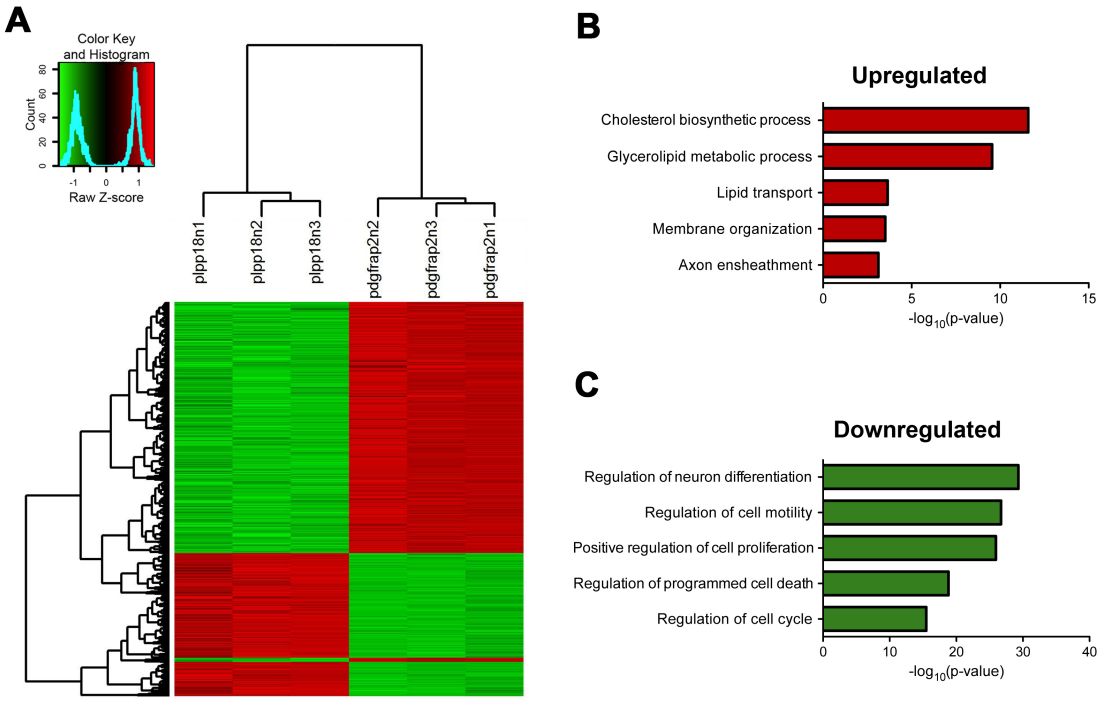


Figure S1. Differential distribution of DNA methylation changes in developing oligodendrocytes. (**A**) Scatter plot of methylation values between each pair of OPC and OL samples revealed high correlation of biological replicates and low correlation between the two stages of development. Numbers denote pair-wise Pearson's correlation scores. Histograms on the diagonal are distribution of percent methylation values for each sample. (**B**) Histogram of the number of hypomethylated and hypermethylated DMRs between OPC and OL samples (distributed by decile DNA methylation differences). (**C**) Distribution of total CpG sites assayed relative to RefSeq gene promoters, exons, introns, and intergenic regions. (**D**) Distribution of hypomethylated and hypermethylated CpG sites relative to RefSeq gene promoters, exons, introns, and intergenic regions (Related to Figure 2).



D

		OPCs enriched genes (3,048)	OLs enriched genes (1,051)	Astrocytes enriched genes (3,457)	Neurons enriched genes (3,514)
RNA-seq during development	Genes upregulated (3,204)	217 (p=1)	652 ($p=6.1e^{-244}$)	431 (p=1)	387 (p=1)
	Genes downregulated (3,247)	1,314 ($p=1.2e^{-243}$)	81 (p=1)	845 ($p=1.4e^{-15}$)	1,159 ($p=4.2e^{-101}$)

Figure S2. Differential transcriptional changes in developing oligodendrocytes. (**A**) Heat-map summary and hierarchical clustering showed clear differences between OPC and OL samples. Only the top 2000 differentially expressed genes are shown. Rows are scaled to an absolute mean of zero (black), with red indicating an increased fold-change and green indicating a decreased-fold change relative to OL. (**B**) Gene ontology analysis of the biological process categories enriched in all upregulated genes. (**C**) Gene ontology analysis of the top biological process categories enriched in all downregulated genes. (**D**) Comparison with Zhang et al., 2014 database showed a higher representation of oligodendrocyte enriched genes and a lower representation of OPC, astrocyte and neuron enriched genes during OL development (Fisher's test) (Related to Figure 2).

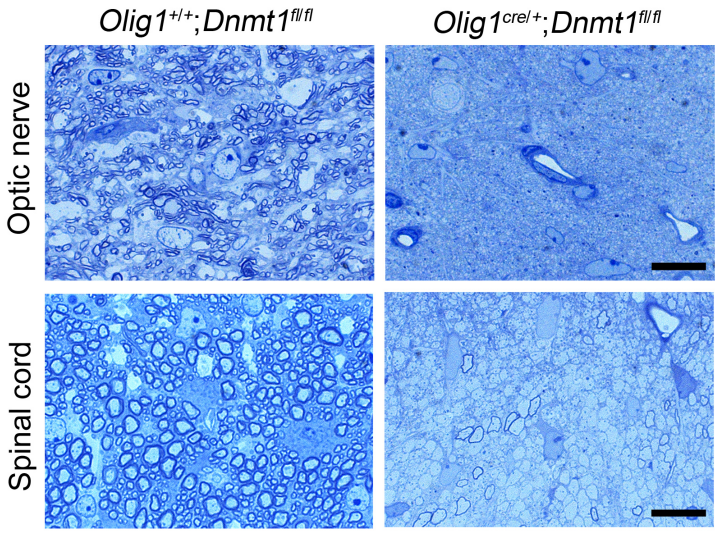


Figure S3. Conditional ablation of DNA methyltransferases in oligodendrocyte lineage cells results in extensive hypomyelination in the central nervous system of *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$ mice. Toluidine blue staining of P16 semi-thin optic nerve and spinal cord sections from *Olig1*$^{+/+}$*;Dnmt1*$^{fl/fl}$ and *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$ mice revealed hypomyelination in the *Dnmt1* mutant mice. Scale bar = 10 μm (Related to Figure 7).

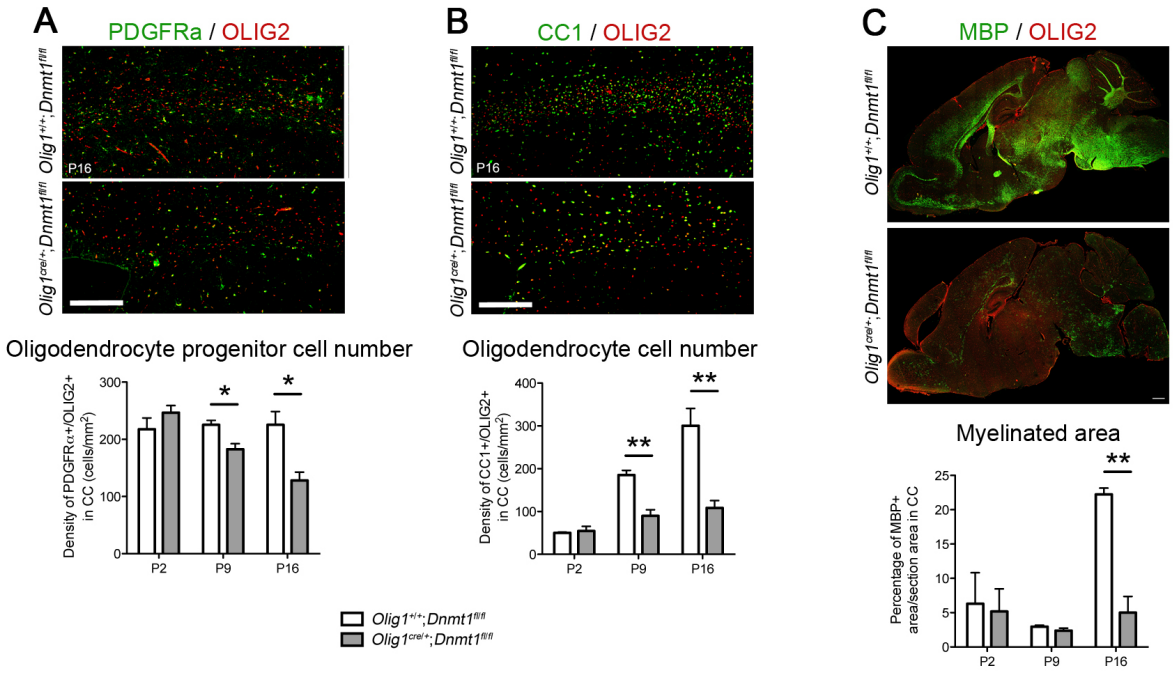


Figure S4. Dysregulation of oligodendrocyte progenitor cell differentiation in *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$*. (**A**). Representative P16 corpus callosum sections of *Olig1$^{+/+}$;Dnmt1$^{fl/fl}$* and *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$* mice, stained for OLIG2 (red) and PDGFR*α* (green) and quantification at P2, P9 and P16. Scale bar = 250 μm. (**B**) Representative P16 corpus callosum sections of *Olig1$^{+/+}$;Dnmt1$^{fl/fl}$* and *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$* mice, stained for OLIG2 (red) and CC1 (green) and quantification at P2, P9 and P16. Scale bar = 250 μm.. (**C**) Representative P16 sagittal brain sections of *Olig1$^{+/+}$;Dnmt1$^{fl/fl}$* and *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$* mice stained for MBP (green) and OLIG2 (red) and quantification of the MBP+ immunoreactive area at P2, P9 and P16, of the corpus callosum. Scale bar = 100 μm. (Related to Figure 5).

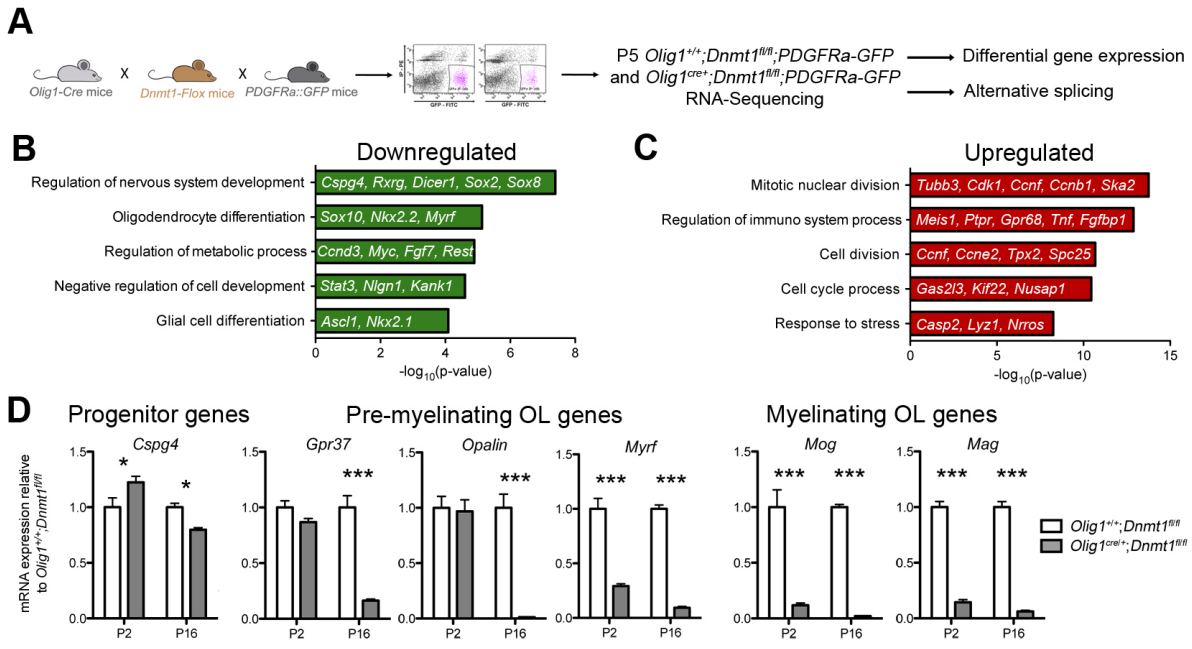


Figure S5. Transcriptomic analysis of *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$ oligodendrocyte progenitor cells reveal differentiation and cell cycle defects. (**A**) Schematic of sorting method for OPC isolation from P5 *Olig1*$^{+/+}$*;Dnmt1*$^{fl/fl}$*;PDGFRα-GFP* and *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$*;PDGFRα-GFP* brains used for RNA-Sequencing and analysis of differential gene expression and alternative splicing. (**B**) Gene ontology of down-regulated genes in *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$*;PDGFRα-GFP* OPC. (**C**) Gene ontology of up-regulated genes in *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$*;PDGFRα-GFP* OPC. (**D**) Quantitative real-time PCR analysis of oligodendroglial genes in P2 and P16 spinal cord of *Olig1*$^{+/+}$*;Dnmt1*$^{fl/fl}$ and *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$ mice. Levels are shown relative to control. Data are mean ± SEM. **p* < 0.05, ***p* < 0.01 and ****p* < 0.005 (ANOVA) (Related to Figure 7).

Supplemental Tables

Sample ID	Cell purification	ERRBS read count	CpGs analyzed	CpGs analyzed (10x min coverage)	Mean CpGs coverage (10x min coverage)	Average bisulfite conversion rate
pdgfrap2n2	*Pdgfra-GFP*	58,863,082	3,322,441	1,464,823	56.5x	99.90%
pdgfrap2n3	*Pdgfra-GFP*	57,071,208	3,934,682	1,466,604	53.9x	99.91%
plpp18n2	*Plp1-GFP*	57,707,358	3,939,894	1,458,478	53.0x	99.70%
plpp18n3	*Plp1-GFP*	59,867,461	3,855,461	1,482,241	55.4x	99.70%

Table S1. Summary of DNA methylation mapping and coverage in developing oligodendrocyte lineage cells isolated by FACS (Related to Figure 2).

Sample ID	Age	Tissue	Cell purification	Mapped read count
pdgfrap2n1	P2	Brain	*Pdgfra-GFP*	130,608,823
pdgfrap2n2	P2	Brain	*Pdgfra-GFP*	133,236,258
pdgfrap2n3	P2	Brain	*Pdgfra-GFP*	134,036,950
plpp18n1	P18	Brain	*Plp1-GFP*	164,310,755
plpp18n2	P18	Brain	*Plp1-GFP*	128,915,819
plpp18n3	P18	Brain	*Plp1-GFP*	143,339,781

Table S2. Summary of RNA-seq metrics for developing oligodendrocyte lineage cells isolated by FACS (Related to Figure 2).

Sample ID	Age	Tissue	Cell purification	Genotype	Mapped read count
Control.OPC_n1	P5	Brain	*Pdgfra-GFP*	*Olig1$^{+/+}$;Dnmt1$^{flox/flox}$*	47,716,304
Control.OPC_n2	P5	Brain	*Pdgfra-GFP*	*Olig1$^{+/+}$;Dnmt1$^{flox/flox}$*	44,807,040
Control.OPC_n3	P5	Brain	*Pdgfra-GFP*	*Olig1$^{+/+}$;Dnmt1$^{flox/flox}$*	43,510,010
Dnmt1.cKO.OPC_n1	P5	Brain	*Pdgfra-GFP*	*Olig1$^{cre/+}$;Dnmt1$^{flox/flox}$*	48,314,684
Dnmt1.cKO.OPC_n2	P5	Brain	*Pdgfra-GFP*	*Olig1$^{cre/+}$;Dnmt1$^{flox/flox}$*	45,483,900
Dnmt1.cKO.OPC_n3	P5	Brain	*Pdgfra-GFP*	*Olig1$^{cre/+}$;Dnmt1$^{flox/flox}$*	43,818,638

Table S3. Summary of RNA-seq metrics for P5 brain OPC isolated by FACS from *Olig1$^{+/+}$;Dnmt1$^{fl/fl}$;Pdgfra-GFP* and *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$;Pdgfra-GFP* mice (Related to Figure 5).

Gene ID	Gene symbol	Log2 Fold change	p-value	q-value
NM_001099299	*Ajap1*	-1.720783522	1.89E-06	0.0016443
NM_016968	*Olig1*	-1.619131128	4.25E-07	0.000454613
NR_002870	*Dnm3os*	-1.564218197	3.22E-05	0.011469382
NM_001289443	*Tnk2*	-1.56067788	9.59E-06	0.004936306
NR_046233	*Rn45s*	-1.547365545	3.65E-05	0.012671414
NM_001081085	*Sapcd2*	-1.510912227	3.74E-06	0.002599731
NM_001040686	*Zfp692*	-1.499321885	1.28E-05	0.005910068
NM_001163098	*Tchh*	-1.49660696	6.63E-05	0.019612237
NM_172736	*Leng8*	-1.478445617	5.09E-05	0.016847324
NR_027059	*2810008D09Rik*	-1.474240496	5.61E-05	0.017738769
NM_023665	*Rsrp1*	-1.471085029	9.07E-06	0.004853302
NM_001013381	*Rsad1*	-1.463802705	6.77E-05	0.019613892
NM_001281955	*Csmd2*	-1.454731936	2.77E-05	0.010152274
NM_009331	*Tcf7*	-1.452309355	0.000104526	0.025954293
NM_011443	*Sox2*	-1.445806221	2.89E-06	0.002229371
NM_183141	*Elfn2*	-1.439542251	7.76E-05	0.021168239
NR_003518	*Pisd-ps3*	-1.434598335	5.39E-05	0.017444801
NM_172633	*Cbln2*	-1.414398981	0.000171633	0.038492797
NM_013661	*Sema5b*	-1.406783914	0.000185656	0.039838152
NM_198305	*Klhl17*	-1.395608517	5.94E-05	0.018346985
NM_027937	*Caskin1*	-1.394825406	1.68E-05	0.006907658
NR_015585	*4933439C10Rik*	-1.385980285	0.00023588	0.044930333
NM_021432	*Nap1l5*	-1.367023596	0.000283731	0.050862481
NM_019684	*Srpk3*	-1.347107084	0.000285313	0.050862481
NM_153537	*Phldb1*	-1.334877217	0.000195769	0.041245059
NM_025869	*Dusp26*	-1.331680772	4.51E-05	0.01529092
NM_013874	*Dpf1*	-1.326740153	9.00E-05	0.023622252
NM_001081391	*Csmd3*	-1.324544229	0.000242821	0.045627318
NM_028408	*Cnih3*	-1.318744889	0.000222147	0.04411979
NM_021387	*Vstm2b*	-1.309767159	0.000186227	0.039838152
NR_104385	*Dab1*	-1.288624327	0.000225279	0.04411979
NM_007893	*E4f1*	-1.27949767	0.000113384	0.027581226
NM_007804	*Cux2*	-1.266459509	9.57E-05	0.024640131
NM_001242411	*Srgap1*	-1.233355805	0.000234658	0.044930333
NM_145382	*Fam193b*	-1.230434342	8.46E-05	0.02261333
NM_001172099	*Cuedc1*	-1.14881556	0.000205909	0.042105291
NM_007984	*Fscn1*	-1.128346205	0.000148285	0.034947485
NM_146019	*Chd3*	-1.096935014	0.000205807	0.042105291
NM_207678	*Ccnl2*	-1.088766902	0.000273162	0.04997787

Table S4. Top 40 genes down-regulated in the *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$;Pdgfra-GFP* OPC (Related to Figure 5).

Gene ID	Gene symbol	Log2 Fold change	p-value	q-value
NR_033123	*4933409K07Rik*	2.905085981	1.09E-15	1.52E-11
NM_001199967	*Gm11127*	2.743176404	1.01E-13	4.67E-10
NM_001278256	*Prnp*	2.710854132	1.04E-14	7.23E-11
NM_001033378	*A430078G23Rik*	2.345393193	2.00E-10	5.56E-07
NR_040518	*Gm4262*	2.233672683	1.14E-09	2.65E-06
NM_001033205	*Zfp575*	2.005551597	4.16E-08	5.78E-05
NM_021281	*Ctss*	1.968077009	1.24E-10	4.29E-07
NM_010130	*Emr1*	1.941351888	1.43E-08	2.48E-05
NM_009987	*Cx3cr1*	1.906089225	1.34E-09	2.67E-06
NM_001038604	*Clec5a*	1.898859987	4.61E-07	0.000457965
NM_009151	*Selplg*	1.883807932	5.92E-07	0.000548648
NM_007574	*C1qc*	1.717900243	2.85E-08	4.41E-05
NM_001282087	*Trpc6*	1.684577186	6.29E-06	0.003898658
NM_008401	*Itgam*	1.672808468	3.51E-07	0.000406832
NM_027571	*P2ry12*	1.672527833	1.75E-07	0.000221359
NM_020261	*Psg23*	1.644693937	1.07E-05	0.005331831
NR_033535	*Gm10845*	1.606693864	7.91E-06	0.004401547
NM_019981	*Tex101*	1.573244933	2.34E-05	0.009041365
NM_011823	*Gpr34*	1.566691549	5.01E-06	0.003316389
NM_011337	*Ccl3*	1.562592331	1.69E-05	0.006907658
NM_001037859	*Csf1r*	1.54452037	2.47E-05	0.009274989
NM_028808	*P2ry13*	1.517101962	3.36E-06	0.002458595
NM_019455	*Hpgds*	1.501743178	2.34E-06	0.001916583
NM_010422	*Hexb*	1.500197439	7.28E-06	0.004218463
NM_019549	*Plek*	1.491674483	1.55E-05	0.006756198
NM_007651	*Cd53*	1.487139907	1.55E-05	0.006756198
NM_007645	*Cd37*	1.472232909	7.30E-05	0.020718576
NM_008879	*Lcp1*	1.449736693	1.13E-05	0.00543756
NM_010745	*Ly86*	1.408452828	6.45E-06	0.003898658
NM_025659	*Abi3*	1.406710078	6.17E-05	0.018651283
NM_008969	*Ptgs1*	1.395616123	1.89E-05	0.007507458
NM_010186	*Fcgr1*	1.370965313	0.000115046	0.027581226
NM_017372	*Lyz2*	1.365040632	0.000161585	0.036833385
NM_030720	*Gpr84*	1.308375746	0.000252735	0.046857079
NM_023695	*Crybb1*	1.301255697	0.00010359	0.025954293
NM_007572	*C1qa*	1.282993294	7.74E-05	0.021168239
NM_010185	*Fcer1g*	1.274720499	0.000155217	0.035971642
NM_172301	*Ccnb1*	1.117143662	0.00021387	0.043099474
NM_009735	*B2m*	1.114538514	0.000184375	0.039838152

Table S5. Top 40 genes up-regulated in the *Olig1$^{cre/+}$;Dnmt1$^{fl/fl}$;Pdgfra-GFP* OPC (Related to Figure 5).

Supplemental Movies

Movie S1. Video of a P13 *Olig1*$^{cre/+}$*;Dnmt1*$^{fl/fl}$ mouse (red mark on the tail) showing tremors, compared to a P13 *Olig1*$^{+/+}$*;Dnmt1*$^{fl/fl}$ mouse (black mark on the tail) (Related to Figure 3).

Supplemental Experimental Procedures

DNA and RNA extraction

DNA and RNA from FAC-sorted cells were isolated simultaneously from the same cell pellet using an AllPrep DNA/RNA Mini Kit (Qiagen) with on-column DNase treatment during the RNA isolation.

Spinal cord tissue RNA was isolated from three biological replicates for each genotype using TRIzol (Invitrogen) extraction and isopropanol precipitation. RNA samples were resuspended in water and further purified with RNeasy columns with on-column DNase treatment (Qiagen).

RNA purity was assessed by measuring the A260/A280 ratio using a NanoDrop, and RNA quality checked using an Agilent 2100 Bioanalyzer (Agilent Technologies).

DNA methylation analysis

The raw sequencing reads were aligned as previously reported (Akalin et al., 2012a). DNA methylation levels were calculated as the ratio of cytosine reads over the total number of sequenced reads for each individual CpG site. Determination of differential methylation was first performed at the single base level, using only CpG dinucleotides with at least 10 sequencing reads and covered in all the samples. The methylKit R package (Akalin et al., 2012b) was used to determine methylation differences between OPC and OL samples and to test for significance of difference by logistic regression. Additional parameters used on top of the default analysis pipeline included: discarding bases that have more than 99.9th percentile of read coverage in each sample to account for PCR bias, normalizing coverage between samples, and merging the read coverage on the forward and reverse strand of a given CpG dinucleotide before doing the test. Regional methylation analysis – with a minimum of 2 CpGs per region – was then performed using the eDMR algorithm (Li et al., 2013), which is based on a bimodal normal distribution model and weighted cost function for regional methylation analysis optimization. The dependence adjustment of the Stouffer-Liptak test was used to combine p-values within a region and a false discovery rate correction was applied to correct for multiple hypothesis testing.

DMRs were annotated on the basis of their position. We defined promoter regions as 10 kb upstream and 2 kb downstream from transcriptional start sites (TSS), exons as RefSeq exons, introns as RefSeq introns, and intergenic regions as those not annotated by the preceding categories, and allowed multiple mapping of DMRs to each category (e.g., if a DMR was 200 bp downstream of a TSS, it would be mapped as both a promoter and exon). As we lacked information for chromatin confirmation or another comparable dataset for region-to-gene mapping, we only assigned gene associations to DMRs residing within promoters, exons, or introns, and annotated the remainder as intergenic DMRs.

MassARRAY EpiTYPER primers

Primers were designed using EpiDesigner software and used to amplify DMRs:

DMR	Forward primer	Reverse primer
Cdc6	TTGAGAGTTATAGGGAAGGGAAAGT	TATAAACCACTAAAACCATCCCAAC
Mcm7_RI	TGTGGTTATTATTATTTTGGGTTTGA GAGGTAGGATAGAGAAAGAAAATAGGGT	ACCCTATTTTCTTTCTCTATCCTACC ATAACTCAAACCACCATCAAAAACA
Meis2	GTTGGATTGTTTGTAAAATGAGTTTTATT	AAAAAATCAAAAAAAACCTCACACC

Forward primers were designed with a 10-mer tag (AGGAAGAGAG) and reverse primers designed with a T7-promoter tag (CAGTAATACGACTCACTATAGGGAGAAGGCT), as per the manufacturer's guidelines.

Quantitative real-time PCR and PCR

For qRT-PCR, RNA was reverse-transcribed with qScript cDNA Supermix (Quanta) and performed using PerfeCTa SYBR Green FastMix, ROX (Quanta), at the Mount Sinai Shared Resource Facility. After normalization to the geometric mean of *Gapdh*, *Pja2*, and *Wdr33*, or *Eef* for ER stress genes, the average values for each transcript were calculated as based on the values obtained in all the samples included for each condition. For PCR, products were run on a 2% agarose gel. A two-tailed Student's t-test or ANOVA was performed to assess statistical differences between the average values in each group at single or multiple time points, respectively.
The following primers were used:

Gene	Forward primer	Reverse primer
Bip	GCCAACTGTAACAATCAAGGTCT	TGACTTCAATCTGGGGAACTC
Cdc6	CGCCTCACCAAGGTACAAGT	CATCCTAAGCCCTAGCTGGC
Cdkn1a	GAGACAACGGCACACTTTGCT	CCACAGGCACCATGTCCAA
Chop	CCACCACACCTGAAAGCAG	TCCTCATACCAGGCTTCCA
Cspg4	ACAGACGCCTTTGTTCTGCT	CCCGAATCATTGTCTGTTCC
Dnmt1	GACAGTGACACCCTTTCAGTTG	GAAGTGAGCCGTGATGGTG
Dnmt3a	CTGGCTCTTTGAGAATGTGG	TGCAGCAGACACTTCTTTGG
Eef	ACACGTAGATTCCGGCAAGT	AGGAGCCCTTTCCCATCTC
Gapdh	ACCCAGAAGACTGTGGATGG	CACATTGGGGGTAGGAACAC
Gfap	GCCACCAGTAACATGCAAGA	CGGCGATAGTCGTTAGCTTC
Gpr37	ACCGGACACAATCTATGTTTTGG	TCTTCCGAGCAGTCACTAGAG
Mag	TGAGACGGAGAGGGAGTTTG	CTCGTCTGGGTGATGTAGCA
Meis2	AGACAAGGACGCAATCTATGG	GCTCGCACTTCTCAAAAACC
Mcm7	AGTATGGGACCCAGTTGGTTC	GCATTCTCGCAAATTGAGTCG
Mog	AAGAGGCAGCAATGGAGTTG	CACAAGTGCGATGAGAGTCAG
Myrf	GGCAGAGCAAGACCAAGAAC	GCACCTTCTGGCACACAGTA
Opalin	TGTTTACCTTGATCCAGCGAAG	CCCCGTGGGTTCATTTCATGT
Pja2	GCCTTGCCATCACTTCTTTC	GCAGATGCGTCAATAACTGC
Pdgfra	TTGGTGCTGTTGGTGATTGT	TCCCATCTGGAGTCGTAAGG
Rbl2	AACTTCCCCATGATTAGCGATG	GGTTAGAACACTGAAGGGCATTT
Wdr33	TGATCTGGTCCCACCAATAG	TGACCAATCGTCTTCCTTCC
Xbp1_S	TCCGCAGCAGGTGCAG	CCAACTTGCCAGAATGCCC
Xbp1_U	GCAGCACTCAGACTATGTG	CCAACTTGCCAGAATGCCC

Immunohistochemistry

Four-micrometer sections were cut, deparaffinized, rehydrated, and washed with PBS plus 0.3% Triton X-100. Antigen retrieval was performed for those antibodies that required it by incubating slides in sub-boiling (94°C) citrate buffer (pH 6.0) for 15 minutes. Slides were incubated in TNB blocking buffer (0.1 M Tris-HCL pH 7.5, 0.15 M NaCL, 0.5% PerkinElmer blocking reagent) for 1 hour at room temperature and then incubated overnight at 4°C with the primary antibodies diluted in the same blocking buffer. After rinsing with PBS plus 0.3%

Triton X-100, sections were processed with the TSA Plus system (PerkinElmer), incubated with the appropriate Alexa Fluor conjugated secondary antibodies (1:200 in TNB blocking buffer, Invitrogen), washed with PBS, and mounted using Fluoromount-G with DAPI.

Immunocytochemistry

After fixation, cells were incubated in blocking buffer (10% normal goat serum in PBS/Triton 0.3%) for 1 hour at room temperature and then incubated overnight at 4°C with the primary antibodies diluted in the same blocking buffer (see list in Supplemental information). Cells were incubated with the appropriate Alexa Fluor conjugated secondary antibodies for 1 hour at room temperature, then mounted using Fluoromount-G with DAPI.

Antibodies

Antibody	References	Experiment
Mouse anti-5mC	Abcam ab10805	Dot blot, IHC (1:200)
Mouse anti-CC1/APC	Millipore OP80	IHC, ICC (1:200)
Rabbit anti-cleaved Caspase 3	Abcam ab3623	IHC (1:200)
Mouse anti-DNMT1	Abcam ab13537	IHC (1:200)
Rabbit anti-DNMT1	Abcam ab19905	IHC (1:200)
Rabbit anti-DNMT3A	Abcam ab2850	IHC (1:200)
Mouse anti-dsDNA	Abcam ab27156	Dot blot (1:200)
Rat anti-GFAP	Invitrogen 13-0300	IHC (1:400)
Rabbit anti-gamma-H2AX	Abcam ab2893	IHC (1:200), ICC (1:400)
Rabbit anti-H3phosphoS10	Abcam ab5176	IHC, ICC (1:100)
Rabbit anti-KI67	Abcam ab15580	IHC, ICC (1:200)
Rabbit anti-MBP	Dako A062301-2	IHC, ICC (1:200)
Rat anti-MBP	Abd Serotec, MCA095	IHC (1:300)
Mouse anti-MNX1	DSHB 81.5C10	IHC (1:20)
Mouse anti-NeuN	Millipore MAB377	IHC (1:200)
Mouse anti-NKX2.2	Gift from Dr. Jessell	IHC (1:20)
Rabbit anti-OLIG2	Millipore AB9610	IHC (1:1,000)
Mouse anti-OLIG2	Millipore MABN50	IHC, ICC (1:200)
Mouse anti-PAX6	Gift from Dr. Krauss	IHC (1:10)
Rat anti-PDGFRa/CD140a	Chemicom CBL1366	IHC (1:500)

Primary rat cell culture and 5-mC Dot blot

After 12-14 days in culture on PDL (5 µg/mL, Sigma-Aldrich) coated flasks, OPCs were isolated via a shake-off procedure (McCarthy and de Vellis, 1980). Contaminating microglia were removed by shaking the flasks at 250 rpm for 1 h at 37°C on an orbital shaker. Subsequently, flasks were shaken at 200 rpm overnight at 37°C. Floating OPC were further purified by differential adhesion. OPC were cultured in defined Sato medium plus PDGF-AA (10 ng/ml) and bFGF (10 ng/ml) on 12-well culture plate for 4 days or for OL the cells were cultured under differentiating condition by withdrawing growth factors while adding T3 for 3 days. Purity of the OPC cultures was routinely assessed by immunocytochemistry for OLIG2 and cultures used were found to be >97% pure. Rat OPC or OL DNA samples were

denatured at 99°C for 5 min, then spotted onto Hybond-N+ nitrocellulose membranes (GE Healthcare). After heating at 80°C for 2 hours, membranes were blocked with 2% non-fat milk in PBT (PBS / 0.1% Tween20) at room temperature for 1 hour, followed by 2 hours incubation with either anti-dsDNA or anti-5mC antibodies at room temperature. Membranes were washed 3 times with PBT, incubated for 1 hour with horseradish peroxidase (HRP)-conjugated antibody, re-washed with PBT, and developed by using DAB HRP substrate kit (Vector labs, SK-4100). Dot blots were scanned and the intensities were measured by ImageJ.

Supplemental References

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